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Physiologically-Based Pharmacokinetic/Toxicokinetic Modeling In Risk Assessment

Peter J. Robinson
Jeffrey M. Gearhart

Alion sciences and Technology Corporation
Dayton, OH

Deirdre A. Mahle
Kyung O. Yu

AFRL/HEPB
2729 R Street, Bldg 837
Wright-Patterson AFB, OH 45433-5707

Elaine A. Merrill

GEOCENTERS
Dayton, OH

Teresa R. Sterner

Operational Technologies, Inc.
1370 N. Fairfield Road, Suite A
Beavercreek, OH 45432

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Air Force Research Laboratory
Human Effectiveness Directorate
Biosciences and Protection Division
Applied Biotechnology Branch
Wright-Patterson AFB, OH 45433-5707

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FOR THE DIRECTOR

//SIGNED//

MARK M. HOFFMAN
Deputy Chief, Biosciences and Protection Division
Air Force Research Laboratory

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**Robinson, Peter J.; **Gearhart, Jeffery M.; Mahle, Deirdre A.; Yu, Kyung O.;
***Merrill, elaine A.; *Sterner, Teresa R.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
* Operational Technologies Corporation
1370 North Fairfield Road, Suite A
Beavercreek, OH 45432

**Alion Science and Technology Corp.
P.O. Box 31009
Dayton, OH 45437

***Geo-Centers, P.O. Box 31009, Dayton, OH 45437

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Physiologically-based pharmacokinetic (PBPK) modeling has become the tool of choice to develop estimates of target site dosimetries in animals and humans for risk assessment purposes. PBPK model compartments correspond directly to the tissues and organs in the species. The drawbacks of PBPK modeling primarily relate to the time, effort and cost involved in appropriately developing, validating and applying a model. We outline some of the practical issues involved in the appropriate development of a PBPK model. Among the first models to be developed and used for risk assessment were those for volatile organics. These basic models are discussed in this report. For some chemicals, however, simpler models are not enough to adequately describe the data. We discuss some of the issues involved in the development of more complex PBPK models. Issues may include more detailed modeling of metabolic processes and specific organs; changes in physiology due to development, pregnancy or aging (life-stage modeling); and interactions between more than one chemical. It may also be necessary to interface the pharmacokinetic models with models of the interaction of the chemical with the target tissue (pharmacodynamic (PD) models) in order to provide a more complete description of the overall process. Certain experimental techniques are central to the successful development of PBPK models. These include methods to experimentally determine blood and tissue partition coefficients, metabolic parameters, and exposure kinetics.

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PREFACE

Technical report development began on 15 June 2004 and was completed on 31 December 2004 under Department of the Air Force Contract No F33615-00-C-6060. Dr. David R. Mattie served as the Contract Technical Monitor for the U.S. Air Force, Air Force Research Laboratory, Applied Biotechnology Branch (AFRL/HEPB) and Dr. Darol Dodd served as Program Manager for the ManTech/GEO-CENTERS Joint Venture Contract (F33615-00-C- 6060). ManTech Environmental Technology, Inc. was acquired by Alion Science and Technology Corporation during this contract. Work was performed under work unit 1710D432 with Dr David R. Mattie as the project director. This work was sponsored by the U.S. Environmental Protection Agency under Interagency Agreement DW-57-93960601-0 with Dr. John Lipscomb, USEPA, ORD/NCEA, Cincinnati, OH, serving as the U.S. Environmental Protection Agency project monitor.

PHYSIOLOGICALLY-BASED PHARMACOKINETIC/TOXICOKINETIC MODELING IN RISK ASSESSMENT

INTRODUCTION

Human health risk assessments are usually based on data collected under less than ideal experimental or clinical conditions. For example, animal studies with exposure to the chemical of interest at relatively high doses over a limited period of time may need to be used to estimate the likely health effects of chronic human exposure to much lower doses, perhaps via a different route of exposure. Although the qualitative relevance of such datasets to the conditions under investigation is usually clear, for example in identifying likely target organs for toxicity, making quantitative use of the data for dose-response analysis and human health risk assessment is often problematic. Historically, this issue has been approached by making use of a series of *uncertainty factors* when extrapolating between animals and humans, high to low doses, from one exposure route to another, etc. (Figure 1). Such factors are used to scale the animal exposure to a value that is considered acceptable to humans under the conditions of interest. Although the use of such factors has become a fine art in the regulatory community, their specific values remain quite arbitrary.

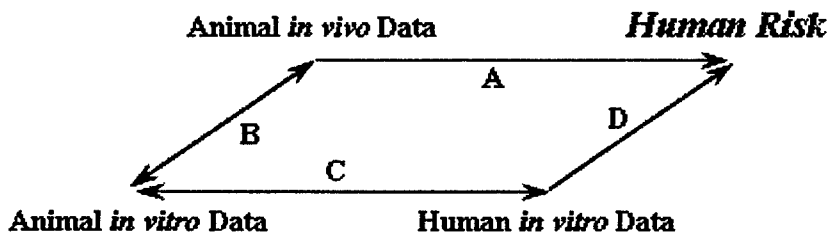

Figure 1. The parallelogram approach. (A) This extrapolation is normally made in human health risk assessments; an uncertainty factor is used. (B) The comparison between *in vivo* and *in vitro* animal data is frequently made. (C) This comparison can be made when *in vitro* systems derived from humans are utilized. (D) Extrapolation from *in vitro* human data, along with extrapolation from *in vivo* animal data, will improve human health risk evaluation.

An alternative approach involves the refinement of the dose-response analysis by focusing on *target site dosimetry*, by mathematically describing the four major processes of pharmacokinetics: bioavailability, distribution, metabolism and elimination. In other words, uncertainty factors for animal to human extrapolation and even human inter-individual extrapolation are replaced with efforts to directly compare the dose of chemical in the target tissue under both the experimental conditions and the human exposure conditions of interest. This comparison is then used to assess the likelihood of health effects in humans from the observed responses of the experimental animals. In contrast to the use of uncertainty or "safety" factors, this approach tries to provide a direct estimate of risk, rather than a conservative upper bound.

Physiologically-based pharmacokinetic (PBPK) modeling has become the tool of choice to develop estimates of target site dosimetries in animals and humans. PBPK models have advantages over more traditional kinetic models in that PBPK compartments correspond directly to the tissues and organs in the species. It is thus possible to meaningfully extrapolate from one animal to another by simply taking into account physiological differences (different organ volumes, blood flows, etc.). The drawbacks of PBPK modeling primarily relate to the time, effort and cost involved in appropriately developing, validating and applying a model for the situation at hand. In this report, we outline some of the practical issues involved in the appropriate development of a PBPK model, so that such costs may be kept to a minimum. The overall process of model formulation, refinement and validation is iterative, as shown in Figure 2.

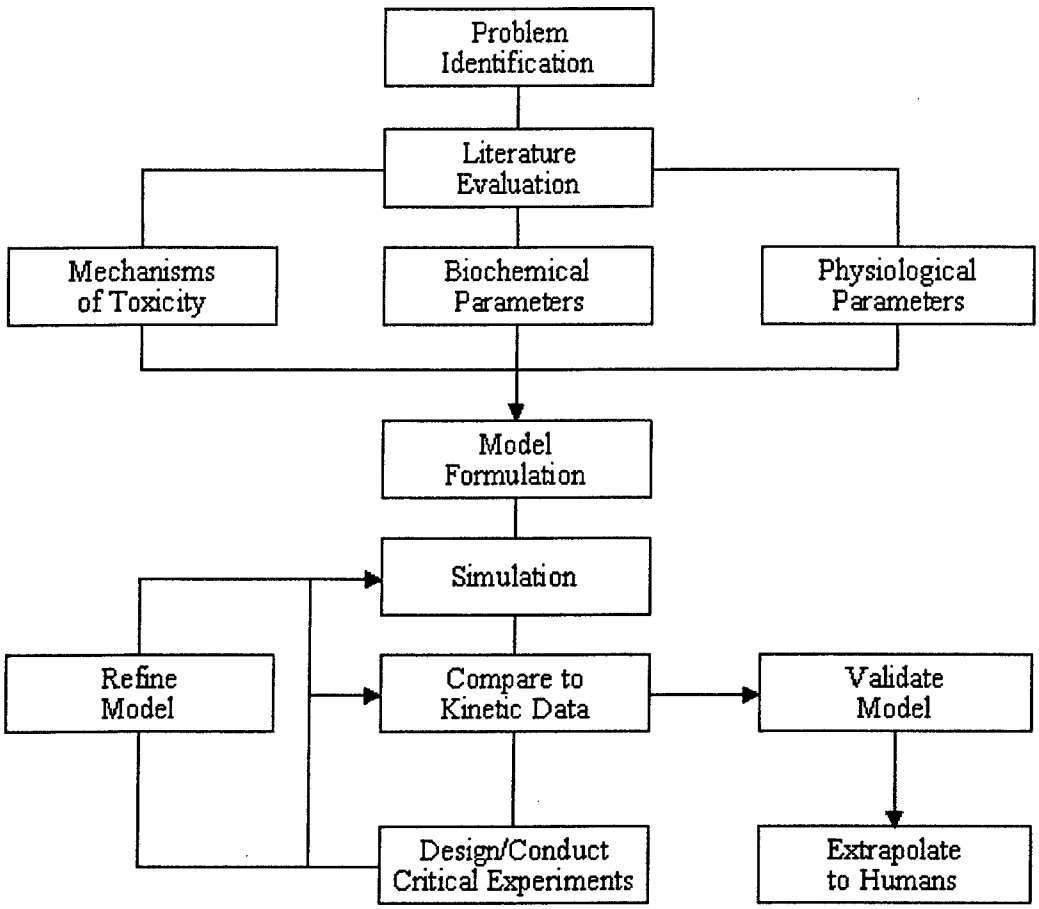

Figure 2. Schematic representation of the model development process in a risk assessment context. Adapted from Clewell and Andersen (1989).

In many cases, PBPK models become complicated and, therefore, pose a risk communication problem. Hopefully the approaches and guidelines presented below will help in the focused development of models that are as simple as possible to explain and extrapolate the data, but not too simple, in that they fail to take into account the appropriate biological processes that underlie a meaningful risk assessment. In terms of their historical development, PBPK models have progressed from the simple to the more complex. Among the first useful models to be developed were those for volatile organics, the so-called Ramsey-Andersen models. Many volatile organic compounds could be modeled to a sufficient degree of physiological detail by making a number of simplifying assumptions (such as using homogenous tissue compartments) (Ramsey and Andersen, 1984). This resulted in a typical "bare bones" PBPK model.

Models of this kind have been successfully developed for styrene, methylene chloride and other compounds (Andersen *et al.*, 1987; Ramsey and Andersen, 1984). These models have been widely used in risk assessment and they, together with the physiological and chemical-specific parameters needed to develop them, are discussed in the next sections.

Certain experimental techniques are central to the successful development of such models. These include methods to experimentally determine blood and tissue partition coefficients (vial equilibration and *in vivo* methods); gas uptake and *in vitro* methods to determine metabolic parameters; and appropriate design of exposure studies to optimize model development and validation, including inhalation (whole-body or nose-only), oral exposure and injection (bolus, infusion) studies.

For some chemicals, however, such bare bones models are not enough to adequately describe the data. In such cases, the underlying assumptions need to be changed and additional biological complexity often is introduced into the model. At this point, a great deal of scientific judgment comes into play in deciding the degree of model complexity that is appropriate. We discuss some of the issues involved in the development of more complex PBPK models in later sections below. Issues may include more detailed modeling of metabolic processes and specific organs, such as the liver and fat; changes in physiology due to development, pregnancy or aging (life-stage modeling); and interactions between more than one chemical. In many cases, it may also be necessary to interface the pharmacokinetic models with models of the interaction of the chemical with the target tissue (pharmacodynamic (PD) models) in order to provide a more complete description of the overall process. We illustrate some of these processes by considering a recently developed PBPK/PD model for perchlorate and iodide in both rats and humans.

Figure 3 shows a schematic for a typical PBPK model for a volatile organic chemical (in this case styrene). As described earlier, PBPK models, as opposed to classical compartmental kinetic models, are physiologically relevant. They include parameters values that quantitatively describe actual biological

systems. These parameter values include physiological and anatomical values such as organ weights and blood flows, and chemical and tissue-specific values such as partition coefficients, measurements of metabolic pathways and transport parameters.

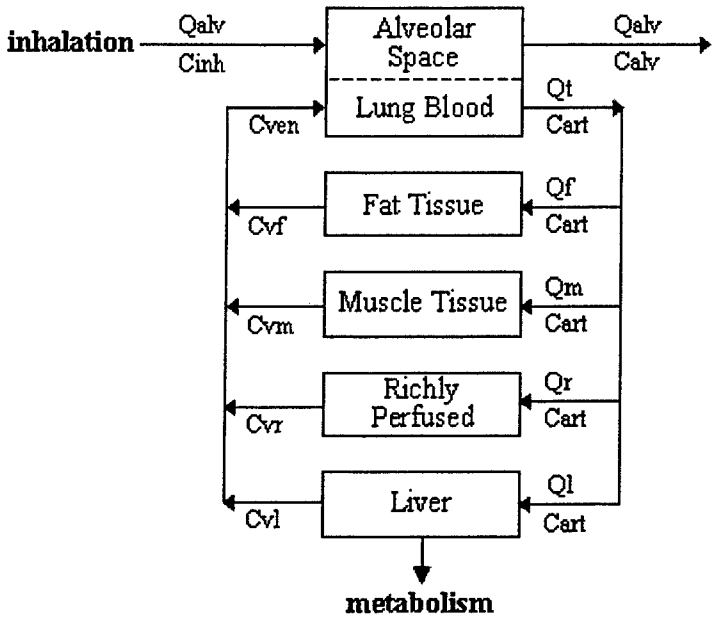

Figure 3. Schematic representation of a simple Ramsey-Andersen PBPK model. Adapted from Ramsey and Andersen (1984).

The mass balance of the chemical in each (non-metabolizing) tissue x, for use in the model, is in general given by

$$V_x\left(\frac{dC_x}{dt}\right) = Q_x\left(C_{art} - \frac{C_x}{(PC)_x}\right) \qquad \text{(Equation 1)}$$

where V_x is the tissue volume, Q_x is the tissue blood flow, C_{art} is the arterial concentration, C_x is the tissue concentration, and $(PC)_x$ is the blood-tissue partition coefficient specific to the chemical, species and tissue.

PHYSIOLOGICAL PARAMETERS

Whenever pharmacokinetic studies are performed for developing a PBPK model, the actual body and organ weights of the animals studied should be used to represent model compartments. Some compartments, which involve active cellular transport mechanisms of the chemical of concern, may require subcompartments; for example, the thyroid compartment in a model may include follicle, lumen and stroma sub-compartments. If possible, actual values of these cellular subcompartments should be used. If such kinetic studies were not performed, organ weights and subcellular compartments can be described as fractions of body weight. This allows automatic adjustment of the compartments with varying body weight. If physiological data are unavailable, several comprehensive lists of physiological parameters for multiple species exist (Arms and Travis, 1988; Brown *et al.*, 1997; Davies and Morris, 1993). Additionally, physiological parameters are generally listed in published PBPK models.

Mean or central tendency values are often used to represent the tissue volumes and blood flows used in PBPK models. However, considerable variability in these parameters may exist for some tissues. For example, blood flow to the stomach can increase tenfold in response to enhanced functional activity (secretion and digestion) (Granger *et al.*, 1985). In such a case, an estimate of resting blood flow may be used (Brown *et al.*, 1997; Davies and Morris, 1993).

Parameters for perinatal and lifetime models need to be considered relative to age-related growth, which varies across tissues. Allometric scaling cannot sufficiently describe the changes in tissue growth, blood flows or fetal/neonatal growth taking place during the perinatal period. As opposed to the typical growth

scenario, organs and tissues cannot be assumed to increase at the same rate in this dynamic system. During gestation, the placenta, uterus, mammary tissue and fetal volume are growing at an accelerated rate in comparison to the other organs. Likewise, during lactation, the mammary gland and maternal and neonatal fat content show the most dramatic changes. Body weight of the neonate also changes dramatically. Maternal weight gain is due mainly to the growth of these select tissues rather than growth of other organs. Therefore, the total change in the maternal body weight can be simply described as the change in these specific tissue volumes added to the initial pre-pregnancy body weight. Likewise, the temporal changes in maternal cardiac output during gestation and lactation can be described as the sum of the initial cardiac output and the change in blood flow to the mammary gland, fat, placenta and uterine tissues.

Growth equations for many perinatal physiological parameters are provided in Gentry *et al.* (2002) and O'Flaherty *et al.* (1992). To date, organ weights and blood flow are not available for early time points in gestation. Clewell *et al.* (2003a) extrapolated to these early period of growth by using a best-fit exponential curve to organ-specific data measured later in gestation.

Significant sex differences in some tissues can also be a source of variability. The distribution of a chemical is affected by multiple factors, including body mass index (BMI), body composition, plasma volume, organ blood flow and the extent of tissue and plasma protein binding of the drug. Women have a higher body fat percentage than men (32.7 to 21.0 percent, respectively) (Brown *et al.*, 1997). Such a large difference would result in disparities in the rate and extent of the chemical's distribution. Women also have a lower average body weight, a smaller average plasma volume and lower average organ blood flows. Gender-specific values are recommended when incorporating a fat compartment, especially for humans. Other major sex differences exist in several protein groups responsible for binding drugs in human plasma. These differences are influenced by concentrations of sex hormones. Sex-based differences in drug metabolism seem to play a greater role in intergender pharmacokinetic variability than

any of the other pharmacokinetic parameters (Gandhi *et al.*, 2003). When sex difference data pertaining to a specific species are not available, values measured from other species, such as mice or rats, can be allometrically scaled as described later.

CHEMICAL-SPECIFIC PARAMETERS

Development of a PBPK model requires certain essential chemical-specific components. These are: 1) experimentally measured or theoretically calculated partition coefficients, 2) an estimate of *in vivo* metabolism, and 3) actual measurements of excretion rates of parent and metabolite. The last is crucial if the metabolite is the toxic moiety and the dosemetric quantity needed for the dose response assessment is not adequately represented by metabolism of the parent.

Partition Coefficients

The partition coefficient (PC) is an essential physiological parameter in a PBPK model. The PC value represents a measure of the solubility of a chemical in a tissue versus a reference matrix, such as blood or air. The PC value quantitatively describes the affinity of a specific chemical for one matrix over another. Variability in a PC value can adversely impact the predictive accuracy of the model. Quite often, in the absence of experimental data, the value of this parameter is estimated based on known physical properties of the chemical in question. Additionally, algorithmic calculations have been developed which predict a chemical specific PC based on experimentally derived PCs from oil and water tissue surrogates (Gargas *et al.*, 1989; Meulenberg and Vijverberg, 2000; Poulin and Krishnan, 1996). Use of these algorithms can be beneficial, particularly when there is a need to reduce costs associated with obtaining tissues from animals. To increase model accuracy, however, chemical and tissue specific PCs should be experimentally determined.

The vial equilibration method is the most common *in vitro* method for determining chemical specific PCs (Gargas *et al.*, 1989; Sato and Nakajima, 1979). This method has been modified to improve reproducibility (Gearhart *et al.*, 1993) and to increase throughput by quantifying multiple chemical PCs as a mixture (Fisher *et al.*, 1997). The vial equilibration method is ideal for volatile and semi-volatile compounds and has been used most successfully for volatile organic solvents. Tissues are harvested from the species of interest, particularly tissues that are the target of the chemical or site of toxicological effect, and incubated with the test compound until equilibrium is reached between the tissue and the headspace. The blood/air or tissue/air PCs are given by the ratio of the concentrations of the chemical in the blood or tissue relative to its concentration in the headspace. A number of operational equations have been derived to calculate these ratios under specific experimental conditions (Fisher *et al.*, 1997; Gargas *et al.*, 1989; Gearhart *et al.*, 1993; Sato and Nakajima, 1979). Time to achieve steady state condition is critical and should be optimized for the test compound. Analysis is done by gas chromatography in a verified linear range. Human tissues can be obtained from tissue bank organizations to provide species specificity to models developed with human data.

To estimate PCs for compounds of low or non-volatility, the method of Jepson *et al.* (1994) can be used. This *in vitro* filtration method has been validated in several biological tissues with compounds having a vapor pressure as low as 9×10^{-6} mm Hg and as high as 14.2 mm Hg. PCs for non-volatile, water soluble compounds can also be determined *in vivo* after establishing steady state concentrations in tissues. While PCs have been estimated *in vivo* after a single dose, infusion of the compound to steady state provides a more accurate estimation of the PC (Chen and Gross, 1979). The *in vivo* PC can be calculated as the ratio C_t/C_B, where C_t denotes the concentration in tissue and C_B denotes concentration in blood, both at steady state. For an eliminating organ such as liver or kidney, this ratio may underestimate the PC value and should be modified to include the effect of blood flow and clearance (Gibaldi, 1982).

When incorporating PC values into PBPK models, it is important to remember that variability between species and between age groups within a species may exist for the same matrix. Aged Sprague-Dawley rats have been shown to have higher PC values for blood, liver, kidney, fat and brain when compared to postnatal rats of the same strain. Another consideration is the effect of freezing on tissue quality and measured PC value. The availability of human tissues from tissue banks has allowed for human-specific PC determination; however, tissues are often shipped frozen and may result in under or over-estimated values (Mahle *et al.*, 2005).

In many cases, the investigator should be prepared to approximate chemical-specific parameters from very limited available data. For example, in a recently developed PBPK model for perchlorate, partition coefficients for the thyroid sub-compartments were based on electrical potentials measured within the thyroid stroma, follicular membrane and lumen after perchlorate (ClO_4^-) dosing (Chow and Woodbury, 1970). Electrical potential differences can be interpreted as effective partition coefficients for charged moieties, such as ClO_4^- and iodide (I^-). These electrical potentials in the thyroid hinder entrance of negatively charged ions from the stroma into the follicle, while the opposite potential from the follicle to the lumen enhances passage of negatively charged species into the lumen and indicates an effective partition coefficient greater than one. Using Chow and Woodbury's measured electrical potentials at the stroma:follicle and follicle:lumen interfaces, effective partitions were calculated (Merrill *et al.*, 2003).

Blood and tissue partition coefficients for a chemical can also be estimated, with varying degrees of success, from its physico-chemical properties such as octanol-water partition coefficients and lipid and water solubilities. In some cases, relevant properties of the blood and tissues, such as lipid composition and binding protein concentrations, are utilized for estimation of partition coefficients. Reviews of various models have been published (e.g., Payne and Kenny (2002)).

METABOLISM: GAS UPTAKE AND *IN VITRO* METHODS

For volatile compounds, the gas uptake technique has been extensively used for determination of *in vivo* metabolism (Filser, 1992; Gargas *et al.*, 1986). In the case of non-volatile chemicals, an approach taken to determine *in vivo* metabolism is to dose animals at varying concentrations, preferably by intravenous injection, and sample the blood over time for parent and/or metabolite concentrations until there is significant disappearance of chemical. Then, with the parameter values for partition coefficients and excretory pathways set at experimentally determined values, Michaelis-Menten parameters, V_{max} and K_m, can be adjusted simultaneously to reproduce the blood concentration time-course of the chemical of interest over the course of the study.

Excretion rates for parent and metabolite are determined by collecting urine, feces and exhaled breath samples from animals dosed at different concentrations and then left in metabolism cages long enough to account for the majority of the mass of parent chemical initially administered. The model parameters responsible for excretion of parent or metabolite are then adjusted to account for the mass of parent and metabolite chemical leaving the body by each route of excretion (Andersen *et al.*, 1984).

Use of *in vitro* results to infer values *in vivo* is central to many of the inferences made in human health risk assessment (Clark *et al.*, 2004; Clewell and Andersen, 1989) (Figure 1). Although there may not always be a direct correspondence between *in vitro* and *in vivo* values, careful consideration must be given before abandoning a measured *in vitro* value in favor of fitting that parameter along with others to the kinetic data as a whole. The latter approach will no doubt produce a better overall fit to the data (and will give an effective value for the parameter that reflects the local biological milieu), but this comes at the expense of the ability to exploit measured differences *in vitro* between animals and humans to reduce uncertainty in the extrapolation.

Allometric Scaling and Interspecies Extrapolation

As previously mentioned, allometric scaling is commonly employed to account for the difference in parameters due to variations in bodyweight (BW) within a given species and across species. Allometry takes the form of power law equations relating parameters to body mass. The classic allometric scaling relationship relating metabolic rate (B) to body mass (M) is:

$$B = B_0 M^{\frac{3}{4}} \qquad \text{(Equation 2)}$$

where B_0 is the normalization parameter or coefficient and M is body mass (Knaak *et al.*, 1995; Lindstedt, 1987). This formula has been extended to a wide range of organisms from the microbes to large vertebrates and plants. Hence, in pharmacokinetics, values for blood flows, diffusion, clearance rates and maximum velocities are most commonly scaled by multiplication with $BW^{3/4}$. It should be noted that body weight in these formulas assumes 'normal' body composition. Further adjustments for the prediction of body size differences clearance and volume in obese or very skinny people will usually require the use of other covariate information (e.g., height, skin thickness) to predict the weight with 'normal' body composition. Additional adjustments may be required to account for developmental changes in very young children (Anderson *et al.* 2000).

MODEL DEVELOPMENT AND VALIDATION

Experimental Design Considerations

To develop a model, one should determine what kind of problems occurs with the chemical(s) of interest. Information on route of exposure, mechanism of target organ toxicity and biochemical and physiological constants can be obtained by searching the literature. The mechanism of target organ toxicity will determine whether the parent compound, metabolite(s) and/or reactive intermediate(s) are responsible for

tissue toxicity. This will help determine whether to investigate a parent chemical and/or metabolite(s) for model development.

Exposure (Administration)

The four major routes of administration of a chemical are inhalation, oral absorption, dermal exposure and intravenous injection. Many conditions of a model, including length of exposure time and concentrations of the chemical, are decided depending upon available kinetic data.

A gas uptake system, as described previously, can be used to expose animals to volatile chemicals in pharmacokinetic studies (Gargas and Andersen, 1988; Gargas *et al.*, 1986). The lungs are the main sites of airborne chemical absorption in either whole body or nose-only exposure and are also elimination sites for some volatile chemicals. Four to five exposure levels should range from lower concentrations (most likely a first order uptake process) to higher concentrations, which should produce saturated kinetics (Michaelis-Menten process). Complications, such as dermal absorption during whole body exposure or ingestion from grooming (licking the fur) following exposure, should be considered if its contribution is significant.

The exchange of gases in the lung, leading to systemic exposure via the inhalation route, is typically described by the following steady-state equation (Krishnan and Andersen, 1994):

$$C_{art} = \frac{Q_{alv}C_{inh} + Q_t C_{ven}}{Q_t + \left(\frac{Q_{alv}}{PB}\right)} \qquad \text{(Equation 3)}$$

where C_{art} and C_{ven} are the arterial and venous blood concentrations (mg/L), Q_{alv} is the alveolar ventilation rate (L/min or L/hr), C_{inh} is the concentration in the inhaled air (mg/L), Q_t is the cardiac output (total

blood flow through the lung, L/min or L/hr) and PB is the blood-air partition coefficient. This equation assumes rapid equilibration of the chemical across the alveolar walls, no significant metabolism in the lung tissue and negligible storage capacity in the lungs. Additional factors that may be taken into account in describing the exchange of gases in the lung include the lung "dead space", in which only 70% of the maximum air capacity of the lung is actually exhaled in any one breath while 30% (the "dead space") remains to be mixed with the next incoming breath; and potential "scrubbing" of chemical in the upper respiratory tract (URT), which prevents it from being immediately absorbed in the lung (though it may be ultimately ingested) (Clewell *et al.*, 2001). The degree of scrubbing varies widely from chemical to chemical and may lead to local effects in the URT (Morris *et al.*, 2004; 2005).

Oral uptake of chemicals by drinking water or bolus administration is a common method of dosing animals and humans with a study chemical. It should be recognized, however, that different species have gastrointestinal tracts with different anatomical and physiological characteristics (Kararli, 1995). The rate of absorption of a chemical into the systematic circulation depends on gastrointestinal (GI) blood flow, GI transit time, surface area, contents, enterohepatic circulation, chemical solubility and the vehicle used. Chemicals can be absorbed by passive diffusion as well as carrier-mediated transport through the intestine epithelium. Enterohepatic circulation can affect uptake and excretion of a compound. When a chemical is absorbed through oral uptake, the first passage through the liver will produce metabolite(s) that are excreted into bile before ever reaching systemic circulation. This first pass will lower the chemical's initial systemic concentration in blood. In addition, some of the chemical will be reabsorbed from bile and result in an increase of parent or metabolite(s) concentration in systemic circulation well after initial uptake.

Oral uptake of a chemical is greatly influenced by vehicle effects on GI absorption. The chemical properties of the vehicles used in the experiments will result in different uptake patterns when compared with consumption of regular food or drinking water, the normal vehicles of environmental exposure.

When absorption of halogenated hydrocarbons in water or corn oil was investigated, for example, the rate of uptake of these chemicals in oil was slower and blood concentrations were lower than those in water (Withey *et al.*, 1983). Vehicle effects, enterohepatic circulation and fasting (versus regular feeding of animals before dosing) are important issues to consider when designing experiments for oral gavage studies. Absorption rate constants of chemicals by oral gavage are obtained by fitting data with a preliminary pharmacokinetic model.

Intravenous (*iv*) injection, by a single bolus or infusion, eliminates the absorption process and introduces chemicals directly into systemic circulation. Unlike the process of oral ingestion, chemicals reach the target organ before first-pass elimination by the liver. Elimination kinetics can easily be obtained by *iv* injection. Water or physiological saline is a frequently used vehicle to dissolve chemicals; oily vehicles should not be used with this route. The constant infusion of a chemical into blood circulation will reach a steady state when the input rate of a chemical is same as its elimination rate and *in vivo* partition coefficients can be measured by analyzing tissue concentrations once steady state is reached.

Distribution (Translocation)

After entering the blood circulation via inhalation, dermal absorption, oral uptake or *iv* injection, a chemical is distributed to tissues throughout the body. Its distribution is related to cardiac output and regional blood flow to the target organs. Richly perfused tissues like liver, kidney, heart and brain will receive the initial chemical distribution. Chemical affinity to tissues then plays a role in determining the storage of a chemical in specific tissues. The free proportion of chemical in blood, not a bound form, generally exerts the toxicological action.

Plasma proteins like albumin may bind with chemicals and act as storage depots. A protein bound chemical becomes unbound when its equilibrium state is changed, releasing the free chemical. This makes the chemical's biological half-life longer, with the blood acting as a storage compartment. Plasma protein

binding alters a chemical's availability for uptake into specific organs and tissues. In general only the free, unbound form is available for uptake and it suffices to have an estimate of the equilibrium free fraction of the chemical in the plasma. However, when uptake is rapid, the equilibrium free fraction may not be a good estimate for the locally free material that is actually available for uptake. In such cases, more detailed data on the kinetics of binding (such as association and dissociation rate constants) may be required to fully characterize the uptake of the material into tissues (e.g., Robinson and Rapoport (1986)).

In addition to plasma protein binding, many organs such as the liver, kidney and bone may also act as storage reservoirs for chemicals, depending on physico-chemical properties and tissue binding affinities. Algorithms have been developed to predict specific tissue distributions of organic chemicals based on the tissue's lipid and water content (Poulin and Krishnan, 1996). Of specific concern, lipophilic compounds are accumulated in adipose tissue and are slowly released to systemic circulation, which may be a major determinant of the time-course of a chemical's concentration in the bloodstream.

Metabolism (Biotransformation)

The liver is the major organ active in metabolizing chemicals. Other tissues such as the kidney, skin, GI tract and lungs also have capacity for metabolism. Mechanisms of action for certain chemicals indicate that some metabolites are more toxic to the target organ than their parent compounds (Brunker *et al.*, 1989; Henningson *et al.*, 1987; Medinsky *et al.*, 1989). Time course studies of the parent compound and/or its metabolite(s) are used to describe uptake, distribution, metabolism and elimination patterns (Clewell and Andersen, 1989). Liver metabolism is presented as a sum of the mathematical descriptions for the first order pathway and/or saturable metabolism, as necessary. Saturable metabolism is usually described in terms of Michaelis-Menten kinetics, so that the metabolism rate (mg/min) is given by:

$$Metabolism\ Rate = V_{\max}\left(\frac{C_l}{C_l + K_m}\right) \qquad \text{(Equation 4)}$$

where V_{max} (mg/min) is the maximum hepatic metabolic rate, K_m (mg/L) is the Michaelis-Menten constant and c (mg/L) is the concentration of the chemical in the liver. The mass balance of the chemical in the liver for use in the model is then given by:

$$V_l\left(\frac{dC_l}{dt}\right) = Q_l\left(C_{art} - \frac{C_l}{(PC)_l}\right) - V_{max}\left(\frac{C_l}{C_l + K_m}\right) \qquad \text{(Equation 5)}$$

where V_l is the liver volume, Q_l is the liver blood flow, C_{art} is the arterial concentration, C_l is the concentration in liver tissue, and $(PC)_l$ is the blood-liver partition coefficient specific to the chemical, species and tissue.

Elimination

An important consideration when developing a PBPK model is the elimination of the parent compound or metabolites through the urine, feces or exhaled air. Biotransformation of xenobiotics often results in more water soluble metabolites that are excreted in urine or feces. After dosing, animals can be maintained in metabolism cages designed to collect urine and feces separately. Analysis for parent compound and metabolites can provide a time-dependent excretion rate. Volatile organic solvents are largely exhaled, especially when the dose route is inhalation. Metabolism of many organic compounds yields CO_2, which is also exhaled. Specialized metabolism cages have been designed to trap exhaled compounds, particularly exhaled CO_2. By quantifying the loss of parent or metabolite through all possible elimination pathways, a mass balance ratio can be achieved.

Parameter Fitting and Optimization

It is not uncommon for the maximum velocity (V_{max}) for the uptake of a specific chemical to vary between tissues and species. Often V_{max} values are not available in the literature and must be derived via

optimization, either visually or with software programs. A visually optimized V_{max} is typically fit to the clearance portion of the time-course data, while keeping all other parameters fixed. The V_{max} is then adjusted so that the model prediction adequately approximates the observed mean. When extrapolating a model from test species to humans, time-course data may be lacking for many compartments due to the difficulty of obtaining human data. In such cases, V_{max} values are estimated to yield kinetics (i.e., tissue:serum concentrations) similar to those described in rats, or whatever species for which the original model was developed. For example, in the perchlorate model, because ClO_4^- data were only available in serum and urine, ClO_4^- V_{max} values for sodium iodide symporter (NIS) containing tissues were scaled from iodide V_{max} values, using the corresponding iodide and ClO_4^- V_{max} ratios established in the male rat model (Merrill *et al.*, 2003).

Diffusion, together with active uptake in particular tissue compartments, can be described using permeability area cross products (PA) (L/hr-kg) and effective partition coefficients. In general, PA values are optimized to the uptake portion of the time course curves prior to setting V_{max} values, with partition coefficients and all other parameters already set (with exception of that compartment's V_{max} value). These visual optimization approaches were used by Merrill and coworkers (2003). The early time-course data represent transfer or uptake of the chemical into a specific compartment.

First order rates describing the loss of a chemical from a compartment, either through metabolism, direct secretion into systemic circulation or elimination out of the body, can be optimized to the clearance portion of the respective data (later time-points), keeping all other parameters fixed. Similarly, reversible binding of a chemical can sometimes be described using an optimized first order rate constant. For example, the binding of a chemical to plasma proteins can be optimized to available serum data (Clewell *et al.*, 2003a; Merrill *et al.*, 2003).

Many investigators believe that model parameterization should be completed with a global optimization of the parameters utilizing all of the data sets used in the model. Several numerical tools exist for this purpose and are relatively easy to use to obtain a global optimum. Many currently available software modeling packages have such software optimization routines (Klein *et al.*, 2002; Levitt, 2002; Tan *et al.*, 2003). Fitting of model parameters to experimental data is often performed by repeatedly using a log likelihood function (Andersen *et al.*, 2001; Collins *et al.*, 1999). The usefulness of such an automated approach varies from case to case and depends on a number of factors, such as the quality of the data and the number of adjustable parameters.

Uncertainty and Variability

PBPK models are designed to reduce the levels of uncertainty typically found in more "classical" risk assessments. Such uncertainty is commonly embodied in the familiar, though quite arbitrary, "uncertainty" or "safety" factors, due to necessary extrapolations across species, doses and routes of exposure. PBPK modeling eliminates the necessity for these factors by providing a quantitative description of the toxicological process that can be used as the basis for these extrapolations. The traditional uncertainty factors are thus replaced with uncertainties associated with the values of the PBPK parameters (uncertainties which are often readily estimated), together with the less well-defined uncertainty associated with the appropriateness of the model itself and its level of abstraction.

Monte-Carlo Analysis and Parameter Sensitivity

Statistical dependencies of model outputs on variations in model inputs are often determined using Monte-Carlo techniques. In these techniques, the effects of parameter variability on the simulation are assessed by assuming a statistical distribution for each parameter of interest. Whenever possible, the actual distribution of a parameter is utilized. Input quantities can be varied by sampling from a number of probability distribution functions, including Uniform, Normal, Exponential, Beta, Chi-Square, F and Gamma distributions. A random value is then chosen from each of these distributions (assuming

statistical independence) and the simulation is run. This process is repeated until a statistical distribution of the output is produced (typically thousands of iterations). The distribution of the model output gives an indication of how much uncertainty (or variability) is due to the uncertainty (or variability) in the values of the input parameters.

If only one input parameter is varied in this way (with the others being held constant), the output variability directly reflects the sensitivity of the system to that specific parameter. Typically the relative impact of each parameter on model predictions is assessed first. This is done after finalizing all model parameters. The model is run at a dose low enough to not saturate its nonlinear metabolic mechanisms for a period long enough to ensure equilibrium. The average biomarker, such as serum concentration area under the curve (AUC), is predicted. The model is then repeatedly rerun, using a 1% increase in each parameter to determine the resulting change in predicted biomarker. Sensitivity coefficients for each parameter are calculated as shown in Equation 6, where A equals the biomarker with 1% increased parameter value, B equals the biomarker using original parameter value, C equals parameter value increased 1% from original value and D equals the original parameter value. In cases where specific parameters drive most of the output variation (i.e., the parameters have the highest sensitivity coefficients), it is particularly important to ensure that an effort is made to define those parameters more precisely than the other, less consequential parameters.

$$Sensitivity\ Coefficient = \frac{\frac{(A-B)}{B}}{\frac{(C-D)}{D}} \qquad \text{(Equation 6)}$$

Model Validation

A major advantage of a PBPK model over classical kinetic compartmental models is that a PBPK model will ideally reproduce the underlying structure and processes of the biological system. In order to see

whether or not this is in fact the case, the model is validated. Once a model has been developed and optimized so that it adequately reproduces the data at hand, it is then required to satisfactorily predict the behavior of the biological system under quite different conditions (i.e., additional studies and data sets). In some cases, particularly where relevant data are scarce, a choice may need to be made as to which portion of data from a study is used for model development and which part is retained for model validation. The most convincing argument for the utility of a model is if it makes clear predictions, which are subsequently verified through the use of different and diverse data sets.

When the model verification process fails, it may mean that the model structure is inappropriate, or that the level of physiological detail in the model needs to be increased (model refinement). In the model development process, decisions have probably been made (based in large part on the investigators' experience) as to what biological processes to include and which to exclude from the model as (probably) irrelevant. At this stage, these decisions need to be reconsidered in order that the model more adequately describes the biological reality. The overall model development process thus takes on an iterative structure as shown in Figure 2.

ADVANCED PBPK MODELS

By their very nature, PBPK models are specific to the compound or compounds in question and need to take into account those processes that are particularly important for those compounds. In many cases, bare-bones models are shown to be inadequate during the model development/validation process. Thus they often become much more complex than the simple Ramsey-Andersen model. In this section, we consider some of the processes that may need to be considered in the development of more complex PBPK models.

Diffusion Limitation

In many cases, the simple description of a compound instantaneously equilibrating between tissue and (venous) blood according to a characteristic partition coefficient (Equation 1) is insufficient to describe the tissue kinetics. One reason for this may be that the tissue compartment is sufficiently large that the material takes a finite time to diffuse from the interface with the blood into the depths of the tissue itself. This may be the case for a lipophilic material distributing (slowly) in the fat or liver compartment, for example. This process can be described with a diffusion term as is traditional in compartmental analysis. Note that such a diffusion limitation term is often expressed in terms of a "PA" or a permeability-surface area product. This term may be misleading as it is usually not suggested that there is an actual membrane barrier to the diffusion process.

PBPK/PD models

These models include a quantitative, mechanism-based description, not just of the kinetic processes (absorption, distribution, metabolism and elimination), but also the relevant interactions of the chemical(s) with the target site(s) and possibly some of the resulting biological responses. There is a natural and gradual transition between what is considered as "kinetic" and what is "dynamic". Many models seamlessly cover both areas in order to describe the biology as a whole. In those cases, a specific biological response is measured as a result of a specific exposure. This response, as well as the kinetics of the exposed material, needs to be modeled in order to provide a complete description of the process and to be able to predict the response under different exposure conditions.

As an example, we consider a recent set of models for the biological effect of perchlorate on thyroid function (Clewell *et al.*, 2003a, 2003b; Merrill *et al.*, 2003). Detection of ClO_4^- in several drinking water sources across the U.S. has lead to public concern over health effects from chronic low-level exposures (Motzer, 2001). Perchlorate inhibits thyroid iodide (I-) uptake at the NIS, thereby disrupting the initial stage of thyroid hormone synthesis. A PBPK model was developed to describe the kinetics and

distribution of both radioactive I^- and ClO_4^- in both humans and rats. The model also simulates the subsequent inhibition of thyroid uptake of radioactive I^- by ClO_4^-, as well as the response of the system to upregulate NIS in the presence of sustained levels of perchlorate. Although thyroid hormones and their regulatory feedback are not incorporated in the model structure, the model's successful prediction of free and bound radioactive I^- and perchlorate's interaction with free radioactive I^- provide a basis for extending the structure to address the complex hypothalamic-pituitary-thyroid feedback system and, ultimately, predict the effects of iodide deficiency and perchlorate exposure. This progressive development of the model structure in order to describe greater levels of detail of the biological system is a major advantage of the PBPK/PD approach to data analysis.

Life Stage Extrapolation

A current concern in the risk assessment community is the particular sensitivity of the developing fetus and newborn to environmental contaminants. In order to address this issue, models of the developing organism need to account for their changing physiology. Physiological parameters can change both as a direct result of growth and changing body-weight (see *Allometric Scaling*) and as a result of various biochemical and physiological processes switching on and off during critical periods of development, maturation and aging (Figure 4).

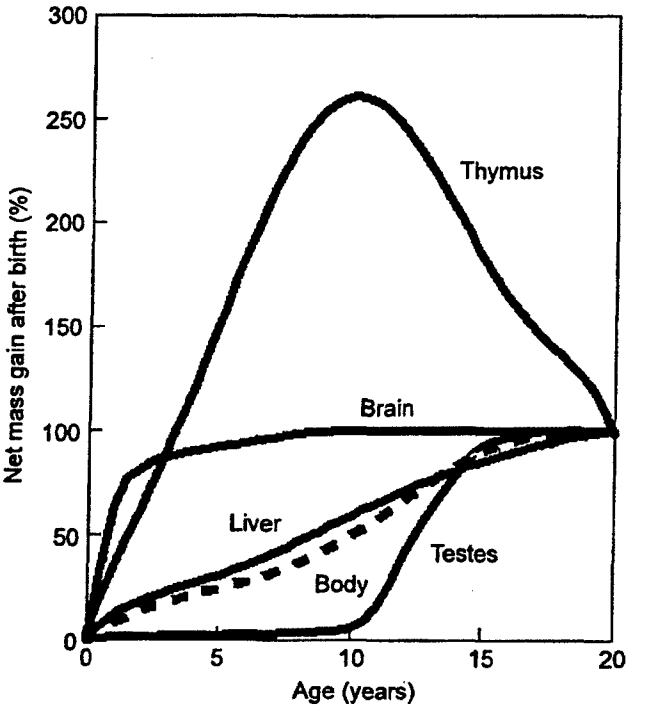

Figure 4. Net mass gain of various body tissues in humans as a function of age. Excerpted from Valentin (ed.) (2002).

Again, the perchlorate models serve as an example. Pregnancy and lactation models were necessary because of the susceptible periods for disruption of thyroid hormone homeostasis during development. These models needed to take into account changes during pregnancy, fetal development and post-natal development. In addition, these models had to incorporate placental and lactational transfer of both perchlorate and iodide (Clewell *et al.*, 2003a, 2003b; Merrill *et al.*, 2003). Figure 5 shows PBPK models for the mother and fetus linked via placental transfer.

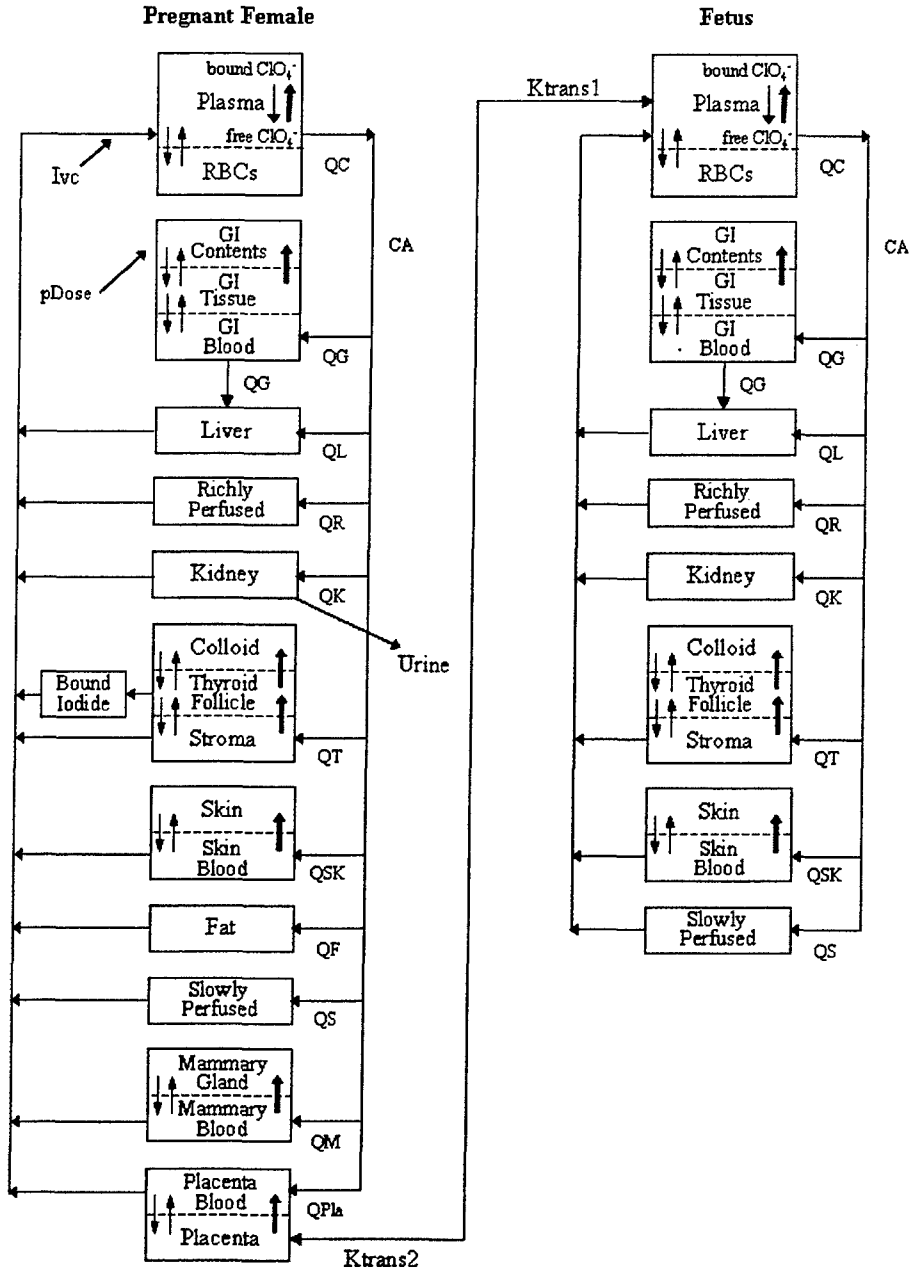

Figure 5. Schematic representation of PBPK model for iodide and perchlorate in the pregnant dam (left) and fetus (right). Adapted from Clewell *et al.*, 2003a.

Additional issues occur in life stage models. When extrapolating life-stage models across species, critical periods may not necessarily "line up". An exhaustive study of the differential timing of neurodevelopmental milestones across mammalian species has recently been conducted by Clancy *et al.* (2001). Some species are born at different developmental stages for different organs. Also, there are

significant physiological changes that take place around birth that must be taken into account in the modeling. In particular, circulatory changes as the newborn begins to breathe by itself are crucial to the model outcome.

Mixtures

Mixtures toxicology is proving to be different from single-chemical toxicology in several fundamental but often unrecognized ways. These differences bring into focus some specific benefits of the PBPK approach to risk assessment issues, even for relatively simple mixtures of just a few components. For example, for mixtures, exposure is nearly always via multiple routes or pathways, which need to be integrated in terms of an absorbed systemic dose. In addition, other stressors (such as noise, heat, infection, etc.) may play a significant role in the overall environmental health response to a mixture exposure scenario. Interactions are potentially many and varied. Pharmacokinetic and pharmacodynamic interactions may occur at the same site or at different sites via complex physiological processes (including defense mechanisms). Cumulative effects of different exposures/stressors over time need to be considered that can alter the "baseline" susceptibility of the individual. PBPK modeling is an important tool used to integrate these processes into a quantitative, predictive framework based on interaction mechanisms.

In addition to the interactions between a relatively small number of interacting chemicals, the study of complex chemical mixtures (such as hydrocarbon fuels) may involve thousands of (frequently unidentified) components, each at very low doses, but together constituting significant exposure levels. In such cases, detailed parameterization of the models is impossible and statistical approaches may need to be used (Robinson and MacDonell, 2004).

The perchlorate/iodide models constitute an example of a simple binary mixture in which the interaction between these two compounds takes place as they compete for transport into the thyroid via the NIS mechanism (Clewell *et al.*, 2003a, 2003b; Merrill *et al.*, 2003). Complex mixtures, however, are usually

considered to contain a dozen or more components. For example, fuels such as gasoline, diesel fuel and jet fuel consist of hundreds or thousands of individual chemical components interacting primarily via competitive metabolic inhibition in the liver. In these cases, it is not possible to model each of the individual binary interactions (the number of parameters would quickly become prohibitive), so alternative methods are needed. Such alternative approaches include "lumping" of chemically similar components, so that they can be represented by a much smaller number of "representative' compounds that are modeled in detail. Alternatively, if the "bad actor" in a complex mixture can be identified (such as benzene in gasoline), the mixture can be modeled as an interacting binary mixture consisting of the "bad actor" and the rest of the mixture lumped together as a single entity (Dennison *et al.*, 2004; Robinson and MacDonell, 2004). The trade-off with this approach is that the lumped component has properties (such as partition coefficients) that may change significantly with time as its individual compounds fare differently in the body as a result of differential uptake, distribution, metabolism or elimination.

CONCLUSIONS: APPLICATION TO RISK ASSESSMENT

In order to use a PBPK model in risk assessment (rather than as a generator of ideas and hypotheses), we must have a degree of confidence in the model. How do we know we have an adequate model? Generally, as a rule of thumb, a "good" PBPK model will:

- Fit or adequately describe diverse data sets, often in multiple species and routes of exposure.
- Use independently estimated (*in vivo*, *in vitro* or even theoretical) values for parameters whenever possible, rather than fitting many parameters to the kinetic data.
- Be physiologically realistic and as simple as possible, with few, if any, ad hoc inclusions.

In general, there is a trade-off between model detail and ability to independently estimate key model parameters.

The primary function of a PBPK model in risk assessment is to provide the basis for reliable extrapolations, usually to humans at low doses via the most appropriate route(s) of exposure. The process of extrapolation raises a number of issues. For example, when extrapolating to low environmental exposures from studies done at relatively high doses, potential issues such as switching from one metabolic pathway to another need to be considered. Such issues are usually taken care of in a good mechanism-based model, but some processes important at low doses may still be overlooked.

Interspecies extrapolation is perhaps the major success story for PBPK modeling. It serves to reduce the uncertainties associated with the extrapolations A and D in Figure 1 by explicitly providing a quantitative framework, based on species-specific physiology, in which to incorporate parameters (physiological, *in vitro* biochemical and theoretical) for both animals and humans. This approach can be extended further by explicitly taking into account physiological and biochemical changes taking place as an organism develops and ages. The standard "parallelogram" approach for interspecies extrapolation can then be extended to a "parallel-piped" approach with extrapolation across both species and life stages using *in vitro* or otherwise independently estimated species- and life-stage-specific parameter values (Figure 6).

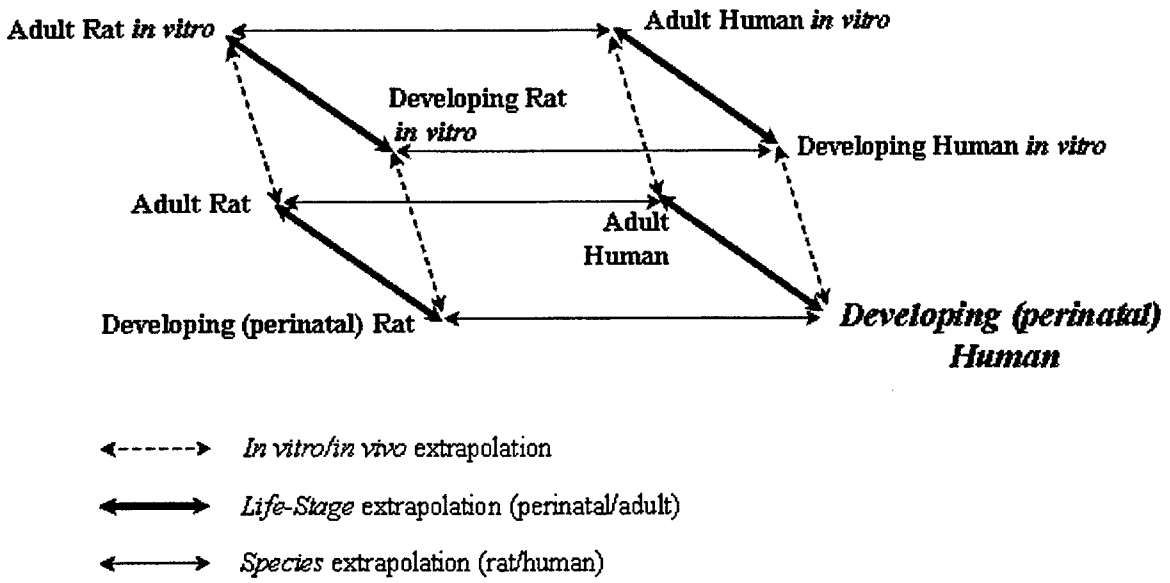

Figure 6. PBPK model extrapolation parallel developed for extrapolation across both species and life stages.

In demonstrating its ability to successfully extrapolate delivered doses across species and routes of exposure, PBPK modeling has been able to replace traditional uncertainty or safety factors in risk assessment calculations. The resulting calculation replaces an approach that in general strives to be conservative (in the sense that the safety factors attempt, above all, to be protective) with an approach that aims wherever possible for accuracy in estimating the delivered dose. This is an important change in philosophical perspective and a word of caution is in order, in that the inevitable remaining uncertainty in the PBPK model, though considerably reduced, is not necessarily on the side of conservatism. It is therefore particularly important to address wherever possible the risk assessment implications of specific uncertainties inherent in any specific PBPK model associated, for example, with parameter estimates. The degree to which particular parameters affect the final outcome of the risk estimation can be explored via sensitivity analysis (Clewell and Andersen, 1989). The propagation of errors and uncertainties in parameter estimates (as well as biological variability), through the model calculations to the final output, is often explored by means of Monte-Carlo analysis (Clewell and Andersen, 1989). What cannot be so easily estimated is the possible error or uncertainty introduced by the particular choice of model structure

consistent with the data and its level of detail; this remains a choice largely based on intuition and experience.

Determination of the delivered dose is not the "end-all" of modeling of the interaction of a chemical with an organism. In many cases, mechanism-based models span the rather arbitrary interface between pharmacokinetics and pharmacodynamics. Such combined pharmacokinetic/pharmacodynamic models are becoming more common and represent a major focus area. Today we have an explosion of information at the cellular level regarding gene expression, protein production and cell signaling. The challenge is to make use of data such as these in a quantitative way, to apply PBPK modeling approaches to the interpretation and integration of genomic, proteomic and metabonomic data, and to apply biochemical network modeling beyond merely identifying relevant networks so as to develop a fully quantitative analysis.

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